

**Effects of aerobic interval exercise training on mouse slow and fast twitch skeletal muscles**

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### Abstract

Aerobic exercise training (AET) is a well-established approach to improve aerobic capacity, cardiovascular function and metabolic efficiency. However, the benefits of exercise have been shown to differ based on the unique modalities of the exercise regimen. We sought to specifically investigate the effects of aerobic interval exercise training on skeletal muscle. For this purpose, 2.5 months old FVB/n male mice (n=6) were subjected to an aerobic interval exercise-training program on a six-lane mouse treadmill for 6 weeks at a maximum speed of 16mts/min/10° incline for 60mins, 5 days/week. Age matched sedentary controls (n=6) were familiarized to the treadmill twice/week at 10mts/min for 10mins. No significant differences were observed in body weight between the sedentary and exercised mice (SED= 30.96±0.656 gms vs EX=31.59±0.610 gms). At the end of the training program, aerobic exercise capacity was determined by subjecting the mice to a graded maximal exercise test on a modular treadmill connected to a CLAMS metabolic unit. Critical markers of whole body aerobic capacity including maxVO<sub>2</sub> measurements (SED=4965.518±139.355 and EX=6295.406±60.71ml/kg/hr) and maximum running speed (SED=33±1.22mts/min; EX=46.8±0.547mts/min) were increased in the trained mice compared to the sedentary controls. Blood lactate levels, measured within 15secs of the exercise test were decreased in trained mice relative to untrained sedentary controls (SED=13.26±0.676 and EX=11.78±0.660mMol/L). Our study indicates that our specific aerobic interval exercise improves overall aerobic capacity indicated by 5.3% increase in VO<sub>2</sub> max and 1.5mMol/L decrease in blood lactate. The Soleus and Plantaris muscles were selected as examples of a slow and fast twitch phenotype respectively. Muscle to body ratios were not significantly different. Fiber type switching in response to the exercise regimen was assessed by Myosin heavy chain (MHC) isoform expression. After 7 weeks of AET, MHC 1 isoform

decreased in Soleus by 16.12% while it increased by 79.77% in the Plantaris compared to untrained sedentary control muscles. On the other hand, MHC IIb increased by 56.72% in Soleus while it decreased 5.92% in the Plantaris after AET. Thus our protocol, which included aspects of both aerobic as well as endurance training, induced shifts in both slow and fast MHC isoforms in the Soleus and Plantaris muscles. Interestingly, we find that the same exercise protocol shifted the Soleus to favor a faster phenotype and the fast Plantaris towards a slower phenotype. The shifts in MHC isoforms in these two muscles studied may indicate the effects of a combined aerobic and endurance demand whereby both muscles may have reached a level of higher efficiency by acquiring a new ratio of slow and fast capabilities.

## Background

***Muscle Fiber:*** A single skeletal muscle is known as muscle fiber or myofiber. Myofibers are made up of many fused undifferentiated mononucleated cells called myoblasts (Kim, 2015). The term “muscle” often refers to a whole muscle such as the Soleus and Plantaris, which are in fact many muscle fibers bound together by connective tissue. The contractions of the skeletal muscle help support and move the skeleton. Skeletal muscles are “striated” because a series of light and dark bands perpendicular to the long axis of fiber can be observed under a light microscope (Widmaier, 2001). This striation is due to the arrangement of numerous thick and thin filaments in the cytoplasm known as myofibrils. Thus, many myofibrils make up myofibers, and bundles of myofibers make up skeletal muscle.

***Basic unit of muscle:*** Sarcomeres are the basic functional units of muscle fibers. A sarcomere consists of one unit of repeating pattern of thick and thin filament along the length of the myofibril (Wayne, 2001). The thick and thin filament is composed of contractile proteins myosin and actin respectively (Wayne, 2001). The binding of actin on the myosin heads in presence of ATP is what allows the muscle to contract (Widmaier, 2001). In addition to actin, the thin filament contains two other proteins—troponin and tropomyosin that play important roles in regulating contraction (McComas, 1993).

***Sliding-filament mechanism:*** The protein myosin, in the thick filament has a projection that extends toward the thin filament called cross bridges (Widmaier, 2001). Sliding-filament mechanism is a process in which during muscle contraction, the cross bridges attaches to the thin filament and exerts force on them leading to overlapping thick and thin filaments (Plowman,

1997). The interaction of the contractile proteins, myosin in the thick filament, and actin in the thin filament is what allows the movement of the cross bridge (Wayne, 2001).

Motor neurons are nerves cells that innervate skeletal muscle fibers. Motor neurons propagate action potential down the axons to the neuromuscular junction (Widmaier, 2001). The neuromuscular junction is where  $\text{Ca}^{2+}$  are released eliciting  $\text{Ca}^{2+}$  release from the sarcoplasmic reticulum (Wayne, 2001). As mentioned before, troponin and tropomyosin are proteins that play important roles in regulating contraction. Specifically, the cross bridges are prevented from binding to actin because tropomyosin molecules partially covering the myosin-binding site (Widmaier, 2001). Troponin is bound to both actin and tropomyosin. When  $\text{Ca}^{2+}$  binds to specific binding sites on troponin, the shape of troponin changes and thus tropomyosin molecules moves away from their blocked positions on actin and exposes the myosin-binding site (Widmaier, 2001). Therefore, these two proteins play an important role for muscle relaxation.

***Skeletal Muscle Fiber Types:*** There are different types of skeletal muscle fibers. Skeletal muscle fibers possess a wide range of structural and functional specializations to support a variety of workloads. They range from slow contracting fibers that are low-powered for endurance based activities to fast contracting fibers that can produce high power for short bursts of high-intensity work. The fibers are categorized based on their mechanical and metabolic characteristics. The type of fiber depends on their maximal velocities of shortening and their major pathway to form ATP (Widmaier, 2001). The maximal velocities of shortening determine whether the fibers are fast or slow fibers (Wayne, 2001). It is dependent on rate of cross bridge cycling, which rely on the maximal rates at which the myosin isozymes split ATP (Widmaier, 2001). Hence, fast fibers

contain myosin with high ATPase activity and slow fibers contain myosin with low ATPase activity.

The enzymatic machinery used to synthesize ATP is another approach to classify skeletal muscle fibers. The energy metabolism used to form ATP determines if the fibers are oxidative or glycolytic fibers (Widmaier, 2001). Oxidative fibers have high capacity for oxidative phosphorylation due to high concentration of mitochondria (Widmaier, 2001). Many capillaries surround oxidative fibers in order to deliver oxygen to the muscle. Oxidative fibers are characterized by a dark-red color due to large amounts of myoglobin. Myoglobin is an oxygen binding protein, which increases the rate of oxygen diffusion within the fiber (Widmaier, 2001). Thus, oxidative fibers are referred to as red muscle fibers. Glycolytic fibers on the other hand use oxygen very minimally compared to oxidative fibers. Hence, glycolytic fibers are referred to as white muscle fibers due to the lack of myoglobin (Wayne, 2001). Instead, glycolytic fibers are characterized by fewer mitochondria, high concentration of glycolytic enzymes, and a large store of glycogen (Widmaier, 2001). Based on these two characteristics, their maximal velocities of shortening and their major pathway to form ATP, skeletal muscle fibers are categorized as slow-twitch oxidative (SO), fast twitch-oxidative (FOG), and fast-twitch glycolytic (FG) (McCommas, 1996).

Skeletal muscle fibers can be classified based on contractile properties, metabolic capacity and more commonly, Myosin heavy chain (MHC) expression. Fibers that express a slow isoform (MHC I) are termed Type I, and those that express any of the 3 fast isoforms (MHCIIa, MHCIIx/d and MHCIIb) as Type IIa, Type IIx/d and Type IIb fibers respectively (Hody 2013). In addition, hybrid fibers such as the Type I/IIa, IIax, IIxb can also exist that express a combination of these MHC isoforms. In humans, Type I, IIa, and IIb fibers have been

described, whereas rodents have four fiber types (I, IIa, IIx/d, and IIb) (Hody 2013). Type I slow-twitch, oxidative fibers are slow in force generation and have an oxidative profile rich in oxidative enzyme expression, mitochondria, and capillary supply (Yan 2010). Type IIa fast-twitch, oxidative fibers are fast in force generation, but have similar oxidative profiles to the Type I fibers (Yan 2010). Type IIx/d fibers are fast-twitch with a glycolytic metabolic profile rich in glycolytic enzyme expression and poor in mitochondria and capillary supply (Yan 2010). Type IIx/d is found in small animals and has an intermediate contractile speed (Hilber, 1999). Type IIb fibers have an even more fast-twitch, glycolytic phenotype than type IIx/d fibers (Yan 2010).

Although fibers are not classified based on their ability to resist fatigue, each of the fiber types have notable differences. Fast-glycolytic fibers are characterized by rapid fatigue, which means contractile activity cannot be maintained for long periods and tension is lost very fast (Widmaier 2001). Slow-oxidative fibers on the other hand are very fatigue resistant (Widmaier 2001). Fatigue resistant means that it can maintain contractile activity for long duration and lose little tension. Fast-oxidative fibers are in between the fast-glycolytic and slow oxidative fatigue resistant spectrum (Widmaier 2001).

**Motor unit:** A motor unit consists of a motor neuron and all of the muscle fibers it innervates. It is important to note that one motor neuron branches to innervate many muscle fibers; however, a muscle fiber is only innervated by a branch from one motor neuron (Widmaier 2001). Although in a single motor unit all muscle fibers are the same fiber type, a whole muscle is composed of motor units of all three types (Widmaier 2001). Hence, each muscle is composed of several types



of myosin heavy chain (Wayne, 2001). Based on the number of each type of muscle fibers there are in a muscle, the muscles' maximal contraction speed, strength and fatigue capacity differ.

***Muscle Adaptation to Exercise:*** Fiber types are extremely plastic and can switch MHC isoforms depending on the demand on the muscle. Specifically, aerobic exercise has been shown to dynamically alter MHC isoform expression, muscle excitation-contraction coupling machinery and energy metabolism pathways corresponding to the mode and duration of exercise.

Aerobic exercise is a term often used to describe exercise that has significant duration but at a relatively low intensity. Running is one example of an aerobic exercise. Endurance capacity increases as the muscle fibers that are recruited adapt to the exercise. Adaptations include increase in the mitochondria and increase in the number of capillaries surrounding the fibers (Widmaier, 2001). Numerous studies have demonstrated other adaptations that endurance training promotes such as fiber type transformation (type IIb/IIc/x to IIa), mitochondrial biogenesis, angiogenesis, and improved insulin sensitivity and metabolic flexibility (Yan 2010). Specifically, studies have shown that endurance training triggers fast to slow fiber type transformation (Hody, 2013).

The proportion of oxidative and glycolytic fibers within a muscle is affected with exercise since the speed at which metabolic enzymes are produced can change. Endurance training can significantly increase the number of fast-oxidative fibers and decrease the number of fast glycolytic fibers within a muscle (Scott, 2001). The type of exercise performed influence the strength and endurance capacity of a muscle. The adaptations that occur at the metabolic level are dependent on training status, intensity, duration and the motor unit involved in the training (Scott, 2001).

***Plantaris and Soleus:*** Plantaris and Soleus are both hindlimb muscles in mouse (Crow, 1982).

Plantaris is a predominantly fast glycolytic muscle while Soleus is a slow oxidative muscle. The slow twitch Soleus contains both slow and fast twitch oxidative type fibers. Fast twitch Plantaris is composed almost exclusively of fast twitch oxidative and glycolytic fast twitch fiber types (Crow, 1982).

***VO<sub>2</sub> max:*** VO<sub>2</sub> max is the volume of oxygen consumption at maximal effort. The more aerobically fit, the higher the VO<sub>2</sub> max. The amount of oxygen consumption increases as exercise intensity increases to produce energy. However, the point at which oxygen consumption doesn't increase with increase in exercise intensity is the VO<sub>2</sub> max. Thus, VO<sub>2</sub> max is measured to quantitate aerobic capacity to determine efficacy of exercise training. Several studies have compared the changes in VO<sub>2</sub> max using interval training involving near-maximal intensity versus continuous exercise of moderate training. These studies found a significantly greater increase in VO<sub>2</sub> in subjects who participated in the interval training (Gormley, 2008). Hence, we found it appropriate to perform interval training with varying speed to maximize the potential changes in VO<sub>2</sub> max.

***Lactate:*** Although lactate is produced in the body all the time, mainly in muscle cells and red blood cells, lactate formation elevates during exercise (Davies, 1996). Lactate forms as a result of carbohydrate breakdown used for energy during anaerobic exercise. Lactate formation and its conversion to pyruvate allow the metabolism of carbohydrates to continue and supply energy. The heart, brain, and slow twitch muscle fibers are major consumers of lactate. Lactate is cleared

from the blood through oxidation by muscle fibers. Blood lactate concentration is determined by the rate of lactate production and clearance. Training has been shown to increase the rate of lactate clearance when blood lactate levels were compared between aerobically trained athletes with untrained individuals (Davies, 1996). Although trained athletes may clear lactate more efficiently than untrained individuals, Stone et al. found that higher levels of blood lactate were measured at the point of failure compared to untrained individuals when performing squats at high intensity. The greater concentration of blood lactate immediately post exercise can be attributed to the greater time and amount of work they performed compared to the untrained individuals. Therefore, Stone suggests that training induces greater tolerance to lactate accumulation (Stone, 1987).

***Fuel source:*** Carbohydrate and fat are the two main sources of energy for muscular metabolism. It is known that during low to moderate intensity exercise, fat is the predominant fuel and increasingly becomes carbohydrate metabolism as intensity increases (Manetta, 2002). As mentioned previously, there are several adaptations that take place with endurance training; one specifically is an increase in the mitochondria. According to Bassett et al. effects of increase in mitochondrial enzymes are 1) endurance trained muscles will oxidize fat at a higher rate and therefore spare muscle glycogen and blood glucose and 2) lactate production decreased during exercise (Bassett, 2000). It is therefore possible that the disparity in  $\text{VO}_2$  max test can be attributed to difference in the mechanism of fuel source that powered muscle contraction during a maximal exercise test between the exercised and sedentary mice. It is important to note that several factors including gender, body composition, exercise mode, and training level influences the pattern of fat oxidation (Chenevière, 2009). The point at which major fuel system changes

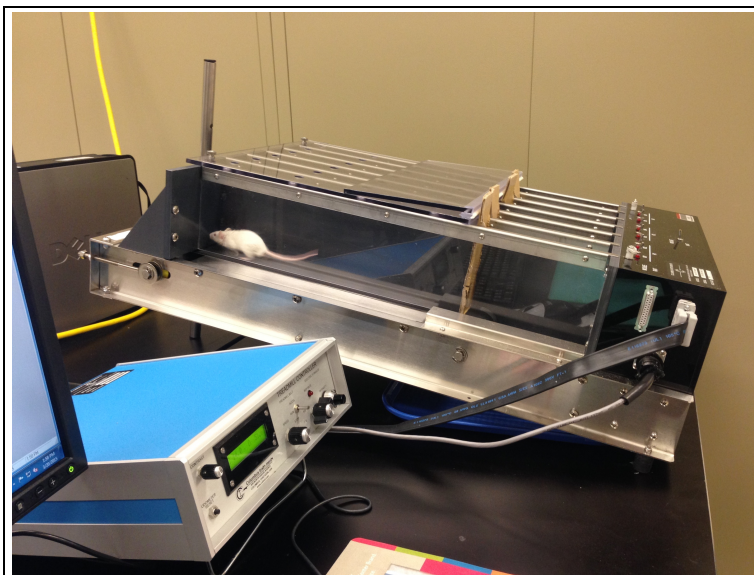
from fat to carbohydrate metabolism during exercise is known as the “crossover point” (Manetta, 2002). After training, the intensity at which this crossover point occurs shifts towards a higher intensity (Manetta, 2002). Therefore, the trained athlete is able to utilize fat for a longer duration than the untrained athlete when performing a matched exercise test. Chenevière et al. defines the exercise intensity, at which the maximal fat oxidation (MFO) rate occurs as fat max. Fat min is defined as the intensity at which the fat oxidation rate reached zero (i.e., respiratory exchange ratio is greater than 1) (Chenevière, 2009). In a cross-sectional study conducted by Venables et al. reported an average fat max occurred at 48% of  $\text{VO}_2$  max compared to 62% of  $\text{VO}_2$  max in moderately trained subjects (Chenevière, 2009). Being able to rely on fat as fuel source is advantageous in several ways. First, fat yields 9kcal of energy per gram versus 4kcal/g of energy for carbohydrate. Second, the body has a seemingly unlimited amount of fat storage, approximating 50-60,000kcal compared to that of glycogen, which has an approximate storage of 1500kcal (Lee, 2014). The ability to predominantly utilize fat for longer period of time is advantageous when it comes to maximal exercise test because it spares glycogen that is needed during higher intensity exercise.

***Previous studies on  $\text{VO}_2$  test:*** Previous study on treadmill training with mice found that with aerobic treadmill training, running for 2h/day 5days/wk, skeletal muscle mass increased by 12-18% (Kemi, 2002). Training consisted of intervals of 8min at 85-90% of  $\text{VO}_2$  max and 2mins at 50%  $\text{VO}_2$  max.  $\text{VO}_2$  max showed an improvement of 49% above sedentary females and 29% improvement in males. This study also found that in a 4-week regimen, myosin heavy chain redistributed toward greater expression of type IIa and IIc/x, but skeletal muscle weight remained unchanged (Kemi, 2002).

Similar to the protocol used for this study, in sedentary mice, treadmill running skills were maintained by treadmill running for 15mins on a flat treadmill at 0.15m/s for 3days/wk.  $\text{VO}_2$  max in untrained mice has been reported to range from 80 to 260 ml/kg/min, and RER at  $\text{VO}_2$  max from 0.91 to 1.28 (Kemi, 2002). Most importantly this study found that the weights of the hind limb EDL and SOL muscles increased substantially (Kemi, 2002).

The purpose of this study was to understand the basic physiological changes in murine Plantaris and Soleus as well as their changes in their overall aerobic capacity from a 7-week treadmill interval training that included both aerobic and endurance components.

## Methods



**Figure 1.** Mice exercised on a 6 lane treadmill

**Animals:** Twelve 2.5months old FVB/n male mice were used for this study. Mice were randomly assigned to either a sedentary (SED; n=6) or exercise (EX; n=6) groups. 6 mice served as controls (sedentary group) with no treadmill interval training. Mice in the exercise group were trained following the aerobic interval

training. Exer 3/6 Treadmill, Columbus Instruments (Figure 1.) was used for training the mice and 1012M-1 Modular Enclosed Metabolic Treadmill for Mice, 1 Lane w/ Shock was used for the exercise performance test in this study.

1. **Acclimatization:** Mice in the exercise group were first acclimatized to a 6 lane small animal treadmill (Columbus Instruments). Mice were acclimatized for a week. Mice were placed in the treadmill for 10mins and allowed to sit as well as explore.
2. **Training:** The mice were trained on the Exer 3/6 Treadmill, Columbus Instruments. A programmed training protocol was used to train the mice with increasing speeds over a period of 7 weeks (Table 1). First week was a sustained easy running at 4, 6, and 8mts/min for 30mins/day, 3days/week. Second week consisted of switching speeds between 8, 10, and 12mts/min for 30mins/day, 3days/week. Weeks 3-8 was interval training at 3mins at 12mts/min and 7mins at 14mts/min for 60min/day, 5days/week at 5-degree incline.

|                                     | DAYS | DURATION | SPEED                               | INCLINE |
|-------------------------------------|------|----------|-------------------------------------|---------|
| <b>WEEK 1<br/>(Acclimatization)</b> | 3    | 30mins   | 4,6 and 8mts/min                    | 0       |
| <b>WEEK 2<br/>(Acclimatization)</b> | 3    | 30mins   | 8,10, and 12mts/min                 | 0       |
| <b>WEEKS 3-8</b>                    | 3    | 60mins   | 3mins@ 12mts/min<br>7mins@14mts/min | 5%      |

Table 1. Aerobic training program

**Sedentary group:** In order to limit the error for the sedentary control group, after the third week of training, the mice were placed in the treadmill for 10mins at 10m/min with 5 degree incline so they have some experience in running prior to the VO<sub>2</sub> max testing. On the 7<sup>th</sup> week of training, the incline was ramped up to 10 degrees.

**Electrical Stimulus:** Electrical stimulus frequency of 1Hz at the lowest intensity was turned on only during the first and second week of training to force the mice onto the moving treadmill belt.

**Exercise Performance Test:** After 7 weeks exercise training, mice were subjected to a graded exercise test on a modular single lane treadmill connected to an OXYMAX/ Comprehensive Lab Animal Monitoring System (CLAMS) setup, to measure  $\text{VO}_2\text{max}$ ,  $\text{VCO}_2$  and Respiratory exchange ratio (RER) by indirect calorimetry in order to assess the aerobic capacity in the exercised and sedentary mice. The mice completed a graded treadmill run to exhaustion on a motorized rodent treadmill with an electric grid at the rear of the treadmill (Columbus Instruments, Columbus, OH). Volume of oxygen consumption ( $\text{VO}_2$ ) during the test was collected every 15 seconds. RER and  $\text{VO}_2$  were monitored throughout the test. Mice performed a 3-min warm-up by walking on the treadmill at 10 m/min and  $0^\circ$  grade. Speed was then increased by 2.0 m/min every 3 min from a starting speed of 10 m/min to a maximum of 40 m/min. The incline progressively increased  $5^\circ$  every stage to a maximum of  $15^\circ$ . Exhaustion was determined as an inability to maintain running speed despite repeated contact with the electric grid. Once mice ran to exhaustion, each mouse was immediately removed from the treadmill and the tail was snipped to measure the blood lactate and glucose using the lactate and glucose strip. The highest volume of oxygen consumed by the mouse during the test was determined as the maximal oxygen consumption ( $\text{VO}_2\text{ max}$ ). This  $\text{VO}_2\text{ max}$  test was performed on both control and trained group. The protocol for the  $\text{VO}_2\text{ max}$  test is shown below with each stage lasting 3mins (Table 2). For every stage, speed and/ or grade was increased.

| Stages  | Speed<br>(mts/min) | Grade<br>(%) |
|---------|--------------------|--------------|
| Warm up | 8                  | 0            |
| 1       | 10                 | 0            |
| 2       | 12                 | 5            |
| 3       | 14                 | 10           |
| 4       | 16                 | 15           |
| 5       | 18                 | 15           |
| 6       | 20                 | 15           |
| 7       | 22                 | 15           |
| 8       | 24                 | 15           |
| 9       | 26                 | 15           |

**Table 2.** VO<sub>2</sub> max protocol

**Body weight:** Every 5<sup>th</sup> training day of the week, body weight was measured for all mice.

**Blood Lactate Measurements:** After the exercise test, the tail tip was sniped and a drop of blood was used to detect lactate levels using a lactate meter (Nova). This measurement was done within 30secs of exercise testing.

**MHC isoform expression by gel electrophoresis:** Soleus and Plantaris muscles were dissected from SED and EX groups and flash frozen in liquid nitrogen. The myosin heavy chain (MHC) isoform composition of homogenates of muscles was determined using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), as in Bicer and Reiser, 2004. The same equivalent wet muscle mass (10.0 µg) was loaded in each 8% acrylamide gel lane so that the amount of MHC in each sample, relative to others, could be determined by densitometry. The gels were silver-stained and the amounts of fast- and slow-type MHC isoforms in each sample



were determined. Comparisons of total MHC protein amounts were limited to samples run on the same gel, due to potential differences in stain intensity between gels. The amount of each MHC isoform in individual samples was determined by using the ImageJ software.

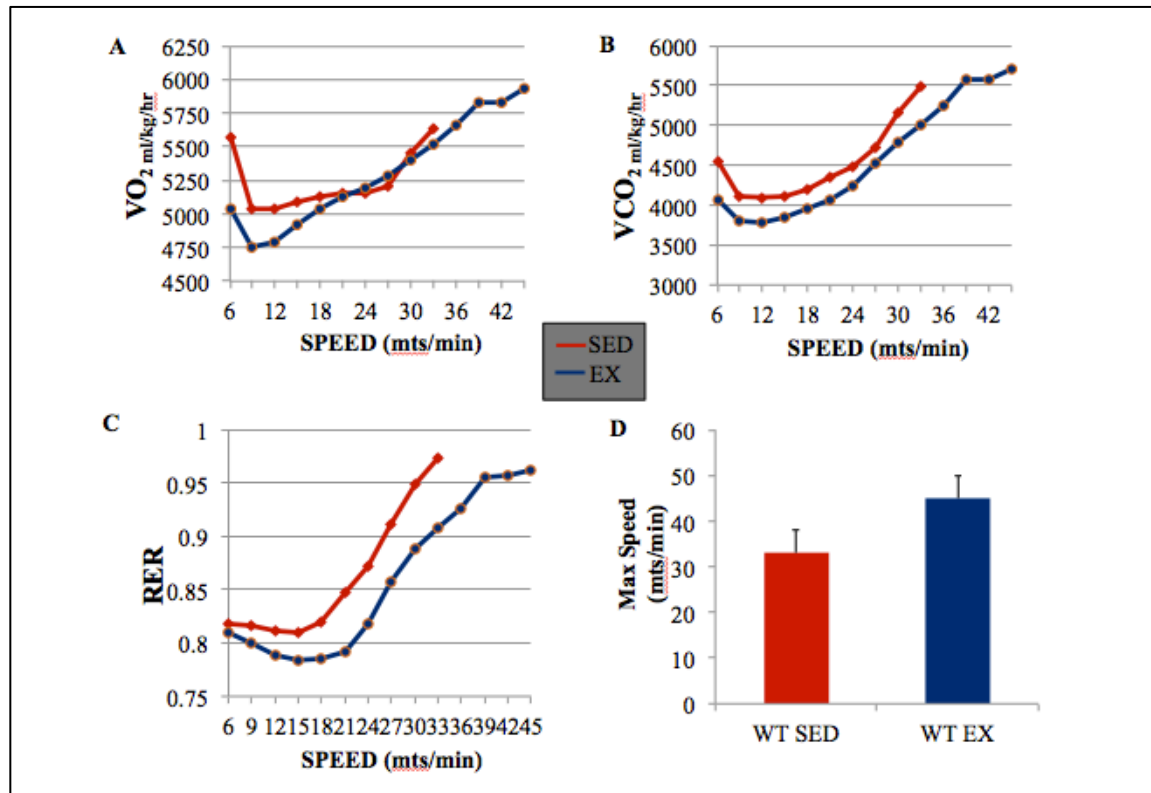
**Statistical Analysis:** Results are presented as mean $\pm$ SE. Group values were tested with a student T-test to determine statistical significance ( $p<0.05$ ).

## Results

All mice successfully completed the treadmill interval training. Training increased the aerobic capacity of trained mice as indicated by the exercise performance test. On average, the exercised mice were also able to run at higher speeds than their sedentary controls (SED=33 $\pm$ 1.22mts/min; EX=46.8 $\pm$ 0.547mts/min). The exercised mice achieved a higher maximal VO<sub>2</sub> and VCO<sub>2</sub> (SED=4965.518 $\pm$ 139.355 and EX=6295.406 $\pm$ 60.71ml/kg/hr) indicating improved aerobic capacity. Figure 2A depicts the relationship between the volume of oxygen consumption, while Figure 2B depicts the relationship between the volume of carbon dioxide exhaled during the exercise test and the speed at which the mice were running in meters per minute respectively. Compared to the sedentary group, the exercise group had a much lower VO<sub>2</sub> during speeds 0 to 20mts/min. We can note that the exercise group was able to continue running at higher speeds (46.8 $\pm$ 0.547mts/min) compared to sedentary mice (33 $\pm$ 1.22mts/min). The longer the duration of time corresponds to being able to exercise at a higher intensity during the max exercise test. Similarly, figure 2B indicates that the volume of carbon dioxide exhaled during the exercise test is lower in the exercise group compared to that of sedentary group. Figure 2C depicts the RER throughout the test, which is the overall ratio between the volume of oxygen consumed and the

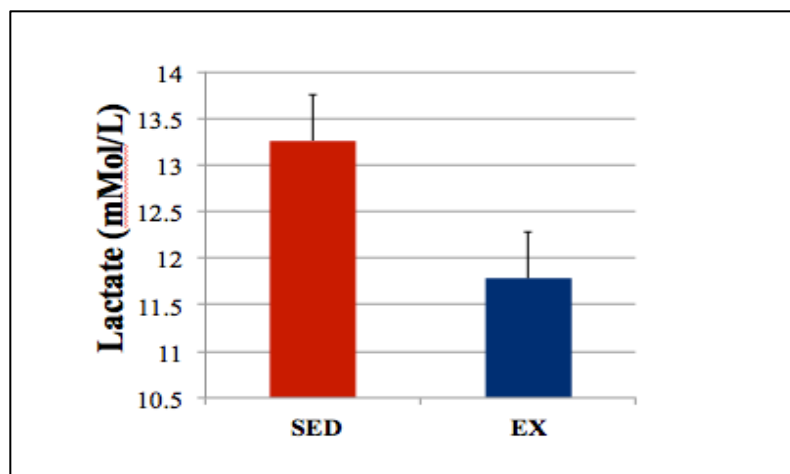
volume of carbon dioxide exhaled. Figure 2C shows a rightward shift of the RER to speed curve indicating aerobic efficiency at lower speeds. It is known that trained subjects have lower RER than sedentary subjects when performing comparable workloads (Jeukendrup, 1997). On average, when the sedentary mice hit their  $\text{VO}_2$  max ( $4965.518 \pm 139.355$ ), their RER was 0.98 compared to an RER of 0.91 at the same  $\text{VO}_2$  for the exercise mice. These data indicate improved oxidative metabolism in the exercised mice. Lower RER exhibited by the exercised mice during submaximal exercise could indicate that fat was the major fuel source through oxidative metabolism compared to the sedentary mice.

In figure 2C we found that the exercised mice has a RER lower than 0.8 during the exercise test period from 9mts/min to 21mts/min. Since Lower RER signifies predominantly fat metabolism, it is possible to reason that the exercised mice were able to recruit fat metabolism longer than did the sedentary controls and could spare glycogen for the later more intense portion of exercise. From these data, we can conclude that our training program produced metabolic changes to improve aerobic fitness of our exercised mice. Figure 2D shows the max speed that the sedentary and exercise group was able to run was notably different running  $33 \pm 1.22$ mts/min and  $46.8 \pm 0.547$ mts/min respectively.

**VO<sub>2</sub> Test:**

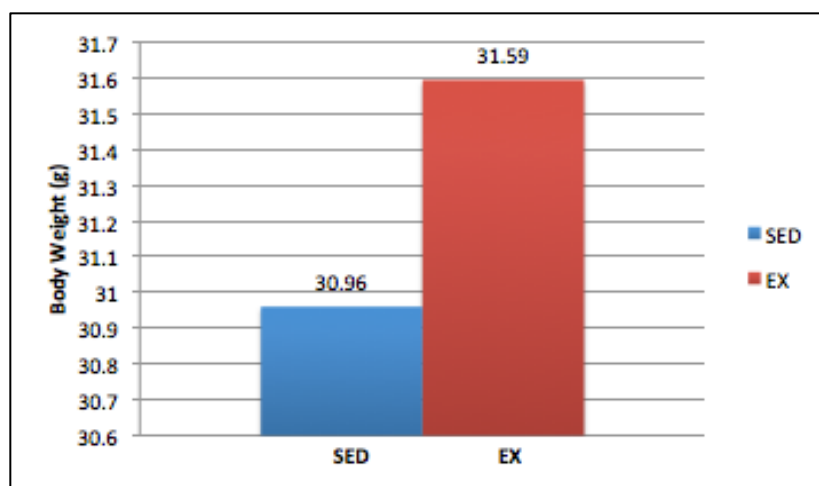
**Figure 2:** (A, B) Aerobic capacity was increased in the exercised mice compared to sedentary mice based on VO<sub>2</sub> and corresponding VCO<sub>2</sub> values that increased linearly with workload intensity. (C) A decrease in the RER values during lower intensity workloads (SED=0.8218 and EX= 0.7874 between 12 and 21mts/min) in exercised mice compared to the sedentary controls indicate improved oxidative metabolism. (D) The exercised mice were also able to run at higher speeds than their sedentary controls (SED=33mts/min and EX=45mts/min).

**Blood Lactate Measurements:** Furthermore, blood lactate level was measured for each mouse post VO<sub>2</sub> max testing. Sedentary group averaged a lactate level of  $13.26 \pm 0.676$  mMol/L while the exercised mice averaged  $11.78 \pm 0.660$  mMol/L (Figure 3). We found that mice trained under our AET protocol had lower lactate levels compared to sedentary mice.

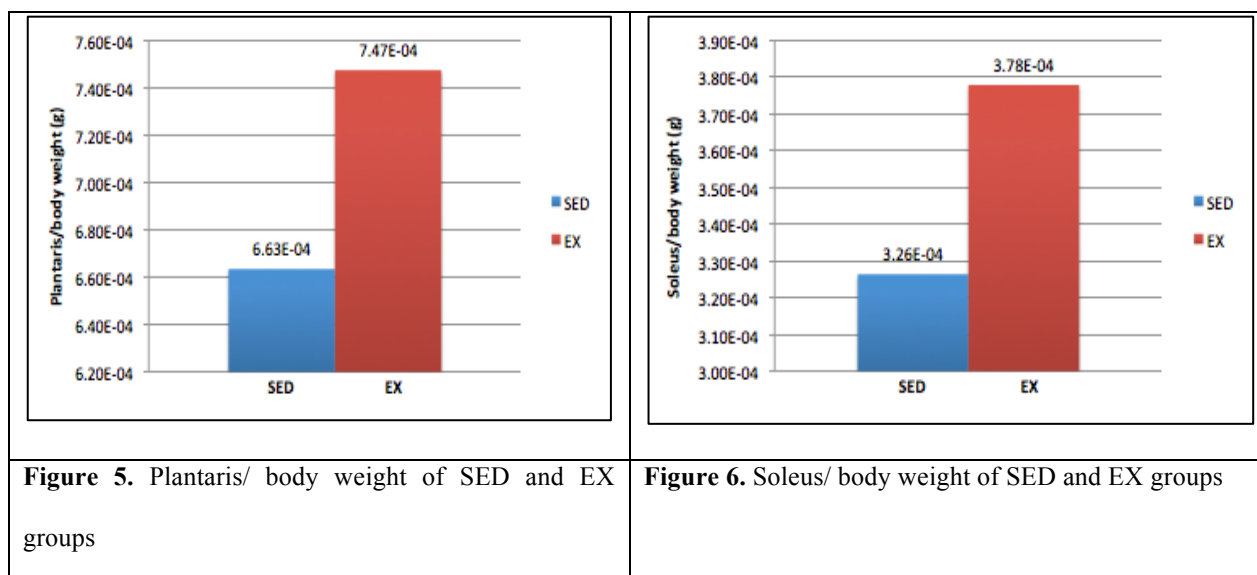


**Figure 3.** Improved oxidative capacity was also associated with decreased blood lactate levels in the exercised mice measured within 30secs after the exercise test.

**Body Weight:** At the end of the training program, the body weights were not significantly different between the SED and EX groups (SED= 30.96±0.656 gms vs EX=31.59±0.610 gms) (Figure 4). The Plantris/ body weight ratio (SED= 6.63E-04±1.40E-04 vs EX= 7.47E-04±3.51E-0.1 ) as well as the Soleus/ bodyweight ratio (SED=3.26E-04±2.52E-05 vs EX=3.78E-04±4.17E-01) were not significantly different between the SED and EX groups (Figure 5 and 6).

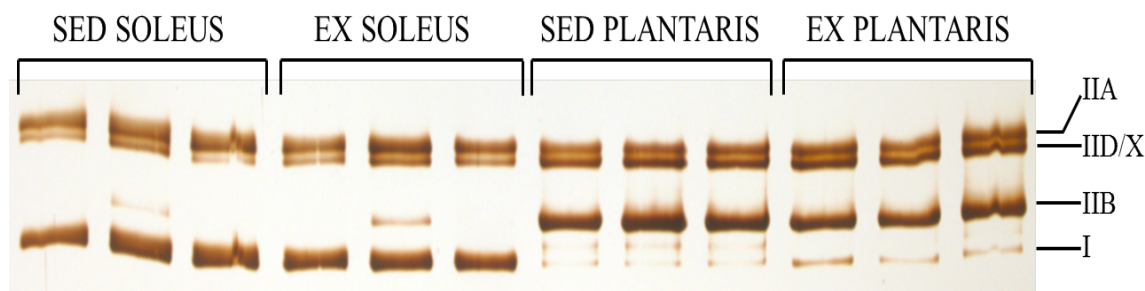


**Figure 4.** Average body weight of SED and EX groups



### Expression of Myosin Heavy Chain Isoforms in exercised slow vs. fast muscles:

After 7 weeks of AET, Soleus (e.g. of slow twitch muscle) and Plantaris (e.g. of fast twitch muscle) muscles were dissected from exercised and sedentary mice, homogenized and subjected to polyacrylamide gel electrophoresis and silver stained to detect MHC isoforms (Figure 7).

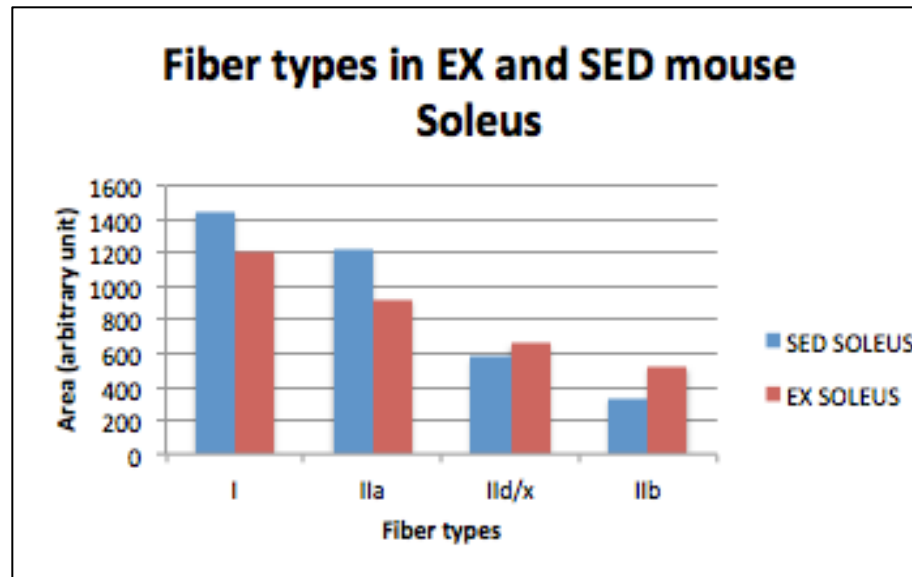


**Figure 7.** MHC isoforms of the Soleus and Plantaris in the SED and EX groups.

MHC isoforms were quantified using the ImageJ software. The density of each MHC isoform band was quantified. The average of 3 samples of Plantaris and Soleus of both sedentary and exercised mice were calculated (Table 3, 4)

|       | SED<br>SOLEUS | EX<br>SOLEUS |
|-------|---------------|--------------|
| I     | 1445          | 1212         |
| Ila   | 1224.7        | 926.0        |
| IId/x | 578.7         | 662.7        |
| Iib   | 335           | 525          |

**Table 3.** Expression of fiber type I, Ila IId/x and Iib in Soleus of sedentary and exercised mice.

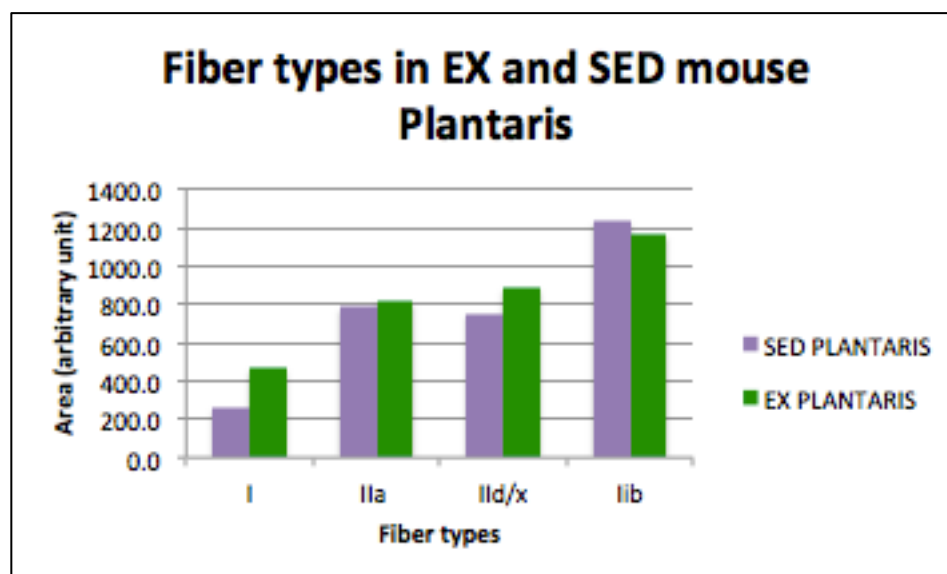


**Figure 8.** The blue and red bars indicate fiber types in the sedentary and exercised mice Soleus muscle samples respectively.

The fiber types (I, Ila, IId/x and Iib) were compared between the SED and EX mice. Results collected from 6 mice (3 SED and 3 EX mice) suggest an overall shift from slower MHC to faster MHC in the Soleus. Specifically, MHC I and Ila expressed in the Soleus decreased while the MHC IId/x and Iib increased.

|       | SED PLANT | EX PLANT |
|-------|-----------|----------|
| I     | 260.3     | 468.0    |
| Ila   | 786.3     | 814.7    |
| IId/x | 754       | 887      |
| Iib   | 1239.3    | 1166.0   |

**Table 4.** Average area of fiber type I, Ila, IId/x and Iib in Plantaris of sedentary and exercised mice.



**Figure 9.** The purple and green bars indicate fiber types in the sedentary and exercised mice Plantaris respectively.

Results collected from 6 mice (3 SED and 3 EX mice) suggest a slight increase in type I MHC and IId/x and slight decrease in MHC Iib. There was no observable change in Ila MHC in the Plantaris between the SED and EX mice. The overall shift in MHCs in the Plantaris is not clear compared to the Soleus.

## Discussion

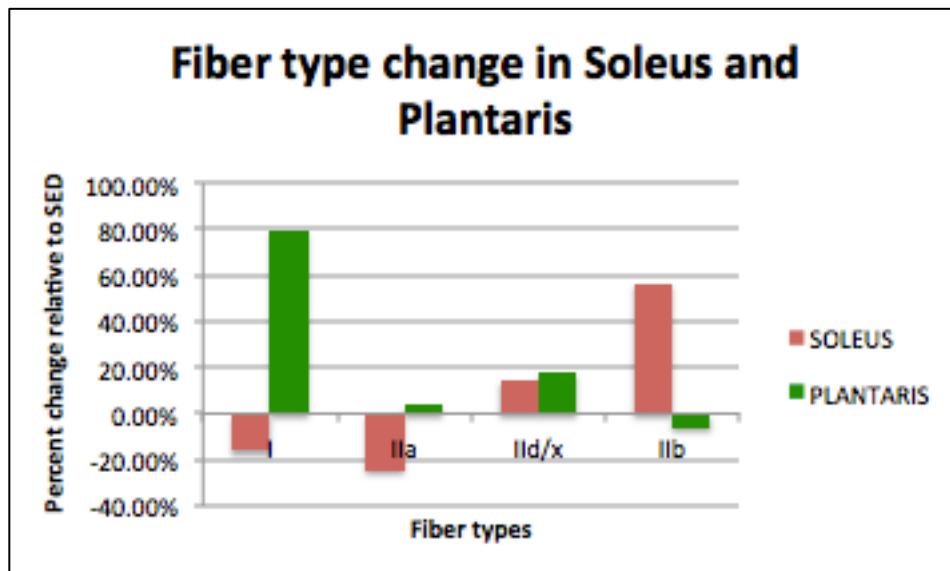
This study aimed to look at the effect of interval treadmill training on fiber type composition of mouse Soleus and Plantaris. Previous studies have found overall increase in the oxidative capacity of skeletal muscle after endurance exercise training (Scott, 2001). However, fiber type transformation between slow-twitch muscle and fast-twitch muscle within the same functional compartment has not been explored. Our study investigated the hindlimb muscles, Soleus, which is predominantly slow-twitch and Plantaris, which is predominantly fast-twitch muscle (Crow, 1982). This study was to understand how each of the fiber types (I, IIa, IIb, and IIx) responds to the treadmill interval training. Fiber type transformation was observed in both Soleus and Plantaris muscles from our interval treadmill training. The treadmill interval exercise training led to different MHC transformation in Soleus compared to Plantaris. Training shifted the Soleus MHC to faster MHC isoforms. MHC I and IIa decreased 16.12% and 24.39% respectively while IIb and IIx increased 14.52% and 56.72% respectively. The Plantaris on the other hand showed a mixed result. The training favored an increase in both MHC I and IIb (79.77% and 17.64% respectively) while it decreased MHC IIa (5.92%). There was a slight increase in the IIa MHC (3.60%) in Plantaris. This study showed that under the same stimulus, changes in the MHC isoforms within the muscle are dependent on the fiber type. Fiber type transformations occur in order to adapt to the stimulus and become more energetically efficient. Therefore, the MHC isoform transformation observed in our study ties back to the type of training we had the mice perform. Our treadmill interval training alternated between periods of high intensity (7mins) followed by a lower intensity running (3mins). Running at faster speeds require fast MHC isoforms. This may have induced a higher recruitment of fast-twitch muscle and so fiber type transformation in the Soleus from slow to a faster MHC isoform is a reasonable explanation in



order to become a faster runner. Previous studies have shown that endurance-training triggers fast to slow fiber type transformation (Yan 2010), which was displayed in the fast-twitch muscle (Plantaris). The Plantaris muscle in the exercised mice showed a 79.77% increase in MHC I while 5.92% decrease in the MHC IIb compared to the sedentary mice. However, more apparent change was revealed in the slower twitch muscle (Soleus), which was the opposite of previous findings. Our interval training demonstrated transformation towards faster MHC isoforms in the Soleus.

|       | SOLEUS  | PLANTARIS |
|-------|---------|-----------|
| I     | -16.12% | 79.77%    |
| Ila   | -24.39% | 3.60%     |
| IId/x | 14.52%  | 17.64%    |
| IIb   | 56.72%  | -5.92%    |

**Table 5.** Percent change of fiber type I, Ila, IId/x and IIb in Soleus and Plantaris.



**Figure 10.** The red and green bars indicate percent changes in fiber types in exercised mice compared to the sedentary mice in the Soleus and Plantaris respectively.

The fiber type composition of Soleus and Plantaris muscles therefore adapted to the exercise in the opposite direction. The slow fiber type (Soleus) favored a faster MHC isoform shift while the fast fiber type (Plantaris) transformed to a higher ratio of slower MHC isoforms. In conclusion, under our treadmill interval training, the expression of MHCs in mouse hindlimb (Soleus and Plantaris) muscles is affected in dissimilar manner. Our study further examined the relationship between the fiber type changes in the exercised mice with increased aerobic capacity seen in the exercise test.

There was a 5.3% increase  $\text{VO}_2$  max in the exercised mice compared to the sedentary mice. The exercised mice were able to handle greater intensity as marked by longer duration, approximately 12mts/min faster, compared to the sedentary mice. The blood lactate level post exercise test was 1.5mMol/L lower in the exercise mice compared to the sedentary mice. Results supported Bassett et al's report that endurance trained mice oxidized fat at a higher rate and therefore spare muscle glycogen and blood glucose, as well as lactate production decreased during exercise. Thus, the observed changes in lactate levels as well as the rightward shift in the  $\text{VO}_2$  levels in the exercised mice could be due to increase in slow fibers and hence in mitochondrial metabolism that predominates in the slow fibers. Our study showed that Plantaris increased in MHC I and IIx/d while it decreased in IIb, which supports the increase in oxidative capacity that are present in oxidative fibers. The Soleus shifted from slow MHC to fast MHC, which seems to contradict the increasing ability of oxidative capacity. However, the higher intensity exercise interval period during the training may have led to favoring change to faster MHC. Although Soleus shifted to faster MHC, because Soleus is still composed predominantly of slow MHC, it still has a high oxidative capacity. We can conclude that our exercise program

was sufficient in generating metabolic adaptations and fiber type transformation to improve physical fitness.

Our study also supports Chenevière et al's conclusion that the after training, the intensity at which this crossover point occurs shifts towards a higher intensity. The trained mice were able to utilize fat for a longer duration than the untrained mice. The ability to metabolize fat and spare glycogen that is needed during higher intensity exercise is beneficial to not only for endurance athletes to maximize performance, but for people trying to lose weight.

Though our research was done on mice that followed a strict diet, this research has potential to help shed light related to exercise and energy metabolism to increase efficiency in training. Through understanding the relationship between training and fiber type transformation in specific muscles, athletes can maximize performance. This study emphasizes the impact of the intensity and duration of the training program on the unique properties of the slow and fast muscle fibers.

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